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Germination responses of *Croton macrostachyus* (Euphorbiaceae) to various physico-chemical pretreatment conditions

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ABSTRACT

Croton macrostachyus Hochst. ex Del. (Euphorbiaceae) is a multipurpose, deciduous, and medium sized tree of pantropic occurrence. Because the species has numerous useful qualities (e.g., establishment and growth in disturbed sites, drought tolerance, fast growth rate, copious litter/necromass production, suitability for agroforestry, and ability to attract avian frugivores), its speedy restoration has become increasingly critical. Germination studies were therefore conducted on seeds pooled from five widely located provenances with a view to supporting efforts geared toward the speedy propagation and restoration of this valuable tree species. Seed pretreatments were achieved using various dilution levels of plant-derived smoke-water (1:1, 1:10, 1:100 and 1:1000), as well as gibberellic acid (GA₃) or potassium nitrate (KNO₃) ranging in concentration from 0.1 to 100 μmol. The control was to use distilled water for seed pretreatment. Seeds were germinated under either illuminated (ca 60 μmol m⁻² s⁻¹; cool-white fluorescent lamp) or non-illuminated conditions. Experiments on the impact of seed storage durations, as well as storage temperatures were also conducted. The study found that germination percentage (GP: ca 90%), and mean germination time (MGT: 14 days) were significantly (P < 0.001) better when seeds were pretreated with smoke-water and germinated under non-illuminated conditions, than when these were pretreated with various concentrations of GA₃ or KNO₃ (GP and MGT of ca 65% and 20 days, respectively). Germination percentage (GP) and germination vigor (GV) declined with increasing storage-time for all storage temperatures, but GV's decline was faster for seeds stored at 22 °C than for those stored at 5 and 15 °C. On the other hand, mean germination time (MGT) increased significantly (P < 0.01) with seed storage-time of up to 8 months at 5, 15, and 22 °C, but the increase was more marked for seeds stored at 22 °C than for those stored at 5 and 15 °C. From these investigations, it is concluded that germination of *C. macrostachyus* seeds through use of smoke-water is faster, cheaper, and technically less demanding, compared to that of either GA₃ or KNO₃. The study also concludes that *C. macrostachyus* is intermediate between orthodox and recalcitrant seeds, and that it is non-photoblastic.

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1. Introduction

Croton macrostachyus Hochst. ex Del. is a multipurpose, medium sized, drought-deciduous pioneer tree that belongs to the Euphorbiaceae, a family that contains large numbers of plant species. It is estimated that there are 8–10 thousand species, contained within 300 genera of the Euphorbiaceae. While Euphorbiaceae is commonly known as the 'spurge' family, *C. macrostachyus* is called 'rushfoil' or 'broad-leaved croton'. The species regenerates naturally in less productive sites including forest edges, mountain slopes and waste grounds under a wide range of ecological conditions (Gilbert, 1995; Negash, 2010).

In Ethiopia, *C. macrostachyus* occurs in regions between 1300 and 2500 m a.s.l (some surveys reporting a range of 500–3400 m a.s.l), with annual rainfall ranging between 750 and 2000 mm. The tree is common in secondary forests, on forest edges, along rivers, around lakes, in moist or dry evergreen upland forests, woodlands, wooded grasslands or clump bushland and along roadsides. It is associated with *Juniperus-Podocarpus* habitats and also occurs in the warmer parts of the montane and semi-tropical rain forests (Friis, 1992; Gilbert, 1995; Negash, 2010). *C. macrostachyus* also grows as a pioneer tree on degraded mountain slopes, disturbed areas, borders of cultivated fields or abandoned cultivation, waste grounds, and along riverine habitats. 'Rushfoil' is, therefore, one of the most widespread tree species, occurring almost throughout the four directions of the Ethiopian landscapes (Negash, 2010). Elsewhere in Africa, *C. macrostachyus* has been reported to occur in Angola, Burundi, Cameroon, Central Africa, Ghana, Guinea, Ivory Coast, Kenya, Malawi, Mozambique, Nigeria, Rwanda, Sudan, Tanzania, Uganda, Zaire, and Zambia (Friis, 1992).

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When not degraded (e.g., through lopping or de-branching), and when grown in the open field, *C. macrostachyus* typically has rounded crown, medium-sized trunk that is studded with relatively long and spreading branches (Negash, 2010). Under the open field conditions, isolated trees are quite short with thick trunks, but can attain heights of over 25 m when growing in fairly crowded forests. According to Negash (2010), one very important morphological and/or developmental attribute responsible for the tree's rapid establishment and growth is the possession (by young trees) of leaf blades that are fairly broad and droopy, collectively covering a space of 360°. Provided that there is no shading by other plants, this type of leaf arrangement and orientation helps the young tree maximize the capture and transformation of sun's light (by means of photosynthesis) throughout the 360° space. This is an important evolutionary adaptation for harvesting as much light energy as possible for photosynthesis during the rainy season, when environmental conditions are favorable for growth. During the dry season, however, this same young tree sheds all of these picturesque leaves, leaving behind only a few thick and highly reduced terminal leaves – another useful adaptation for coping with water scarcity by disposing off of surfaces with large numbers of stomata (Negash, 2010).

Owing to its useful biological traits as a fast growing, drought-deciduous tree, *C. macrostachyus* is essential for soil regeneration and water conservation; two fundamental processes critical for sustainable agriculture, food security and livelihood maintenance in developing countries such as Ethiopia. The species is competitive; with distinctive morphological and physiological characteristics that include rapid production of large numbers of leaves and flowers during the rainy season and shedding these during the dry season. The tree is quite persistent, regenerating large numbers of coppices or shoots, even when it is repeatedly lopped or degraded (Negash, 2010). Provided that environmental and soil conditions are favorable, *C. macrostachyus* does establish well and can grow quite fast on reasonably good and well-drained soils, but prefers red or loam soils to vertisols (Negash, 2010). The latter soils are known for their shrink-swell properties (during the dry and wet seasons, respectively), and for getting waterlogged during the rainy season.

Very unfortunately, natural regeneration of *C. macrostachyus* in Ethiopia has been declining owing to the long-standing, rampant, and disruptive anthropocentric activities (including deforestation, agricultural land expansion, and individual tree degradation) (Negash, 2010). In the dry Afromontane settings of Ethiopia, such disruptive activities invariably lead to altered local climatic and soil fertility conditions.

Clearly, successful restoration of *C. macrostachyus* is predicated not only on provenance selection, domestication, and propagation, but also on a fairly sound understanding of species' seed behavior and germination physiology.

Seed physiological studies allow the understanding of: (1) factors which regulate seed longevity under storage conditions; (2) requisite physico-chemical conditions for germination; and, (3) successful establishment of the resulting germinants under nursery and field conditions (Bewley and Black, 1994; Bradbeer, 1988; Negash, 2010; Yang et al., 2005). In tropical settings, where significant numbers of important tree species are known to produce either recalcitrant or intermediate seeds, understanding of their seed biological characteristics, germination physiology, and impacts of storage-time and temperature on seed viability and germination responses are critical for provenance

selection, domestication, propagation and cultivation (Berjak and Pammenter, 2008; Negash, 1995, 2003, 2004, 2010; Xia et al., 2012).

The publication by De Lange and Boucher in 1990 underscored the importance of plant-derived smoke–water in increasing seed germination (and also improving subsequent seedling development). Interestingly, as early as 1966 Wicklow (quoted in Wicklow, 1977) noted that the post-fire chaparral annual *Emmenanthe penduliflora* Benth. (Hydrophyllaceae) “– was locally abundant on burned areas formerly occupied by windrows of piled brush, but not in the zones between these rows”. Subsequent to this observation, Wicklow (1977) concluded that “–the charred remains of chaparral vegetation serve as a germination trigger” for *E. penduliflora*.

Relatively large numbers of studies have since been conducted with a view to applying the technology, as well as understanding the underlying mechanism(s) for smoke–water-stimulated seed germination of diverse plant species (e.g., Brown et al., 2003; Chumpookam et al., 2012; Crosti et al., 2006; Daws et al., 2008; Demir et al., 2012; Ghebrehiwot et al., 2011; Light et al., 2009; Roche et al., 1997; Van Staden et al., 1995, 2000; Zhou et al., 2012).

The interaction of light, GA₃ (gibberellic acid 3) and KNO₃ (potassium nitrate) has been reported by a number of authors (e.g., Alboresi et al., 2005; Hartmann et al., 1997; Jovanovic et al., 2005; Plummer and Bell, 1995; Taiz and Zeiger, 1998). It had also been noted that GA₃ or KNO₃ become effective germination-promoting agents when combined with light and appropriate temperatures (Bewley and Black, 1994; Thanos and Rundel, 1995). Further, it is known that seed vigor and viability decline more rapidly when seeds are stored at higher than at lower temperatures (Bewley and Black, 1994; Bradbeer, 1988; Roberts, 1961).

The objective of the present study was, therefore, to examine germination responses of *C. macrostachyus* by using pooled seed provenances collected from five ecologically diverse regions of Ethiopia. Specifically, the study aimed at subjecting seeds to different physico-chemical pre-treatment regimes and germinating these under illuminated and non-illuminated, as well as under laboratory and nursery conditions.

2. Materials and methods

2.1. Seed/fruit collection and processing

Mature seeds/fruits were collected from trees sampled from five ecologically varied regions of central, central-eastern, central-western, and western Ethiopia (Table 1).

The first batch of collection was made from west Arssi (central Ethiopia), while the last batch of collection was secured from Jimma (central-western Ethiopia). A minimum of five widely spaced trees at varying stages of mast-fruiting were sampled from each of the localities shown in Table 1; and the collection process covered all parts of the trees' crowns with mature fruits. Also, seeds were collected from fruits that had just dehisced (i.e., right before the seeds became scattered onto the ground). Perforated polyethylene bags were used to collect the fruits and/or the seeds. Soon after collection, the bags containing the fruits and/or seeds were packed in perforated sacks and transported to the Plant Physiology Laboratory of the Plant Biology and Biodiversity Management Department (College of Natural Sciences, Addis Ababa University). Seeds/fruits were allowed to air-dry on laboratory benches for one week at room temperature. Seeds from dehiscent fruits were

Table 1

Croton macrostachyus provenances, names of localities from where seeds were collected, and the corresponding geographic locations.

Provenance	Locality	Latitude	Longitude	Altitude
West Arsi	Shashamané	7° 08.3'–7° 49.0' N	38° 37.9'–38° 42.8' E	1740–1850 m
West Shoa	Bako	8° 57.4'–8° 59.8' N	37° 10.3'–37° 22.8' E	1595–1750 m
East Wollega	Gidda Ayana	9° 44.8'–10° 55.3' N	36° 03.9'–36° 45.4' E	2100–2425 m
Illu-Ababora	Bedelie	8° 03.2'–8° 20.9' N	36° 16.3'–36° 64.0' E	1980–2350 m
Jimma	Mana	7° 46.0'–7° 47.2' N	37° 03.0'–37° 39.1' E	1995–2190 m

extracted by hand, and were allowed to air-dry for additional 48 h on the same bench under the same laboratory conditions. One kg of each of the five provenances were then pooled and stored at 5, 10, 15 and 22 °C until used for subsequent experiments.

2.2. Smoke–water preparation

Smoke–water was prepared by burning 200–250 g of small branches and leaves of various plants in a beekeeper's smoker (diameter, 100 mm; depth, 200 mm). Among the plants used for smoke–water preparation were *C. macrostachyus*, *Juniperus procera* Hochst. ex Endl. and *Milletia ferruginea* (Hochst.) Bak.]. The generated smoke was forced into a 250 ml Erlenmeyer flask (E-flask) containing 200 ml of double distilled water. This was achieved by means of a plastic hose fitted to the mouth of the beekeeper's smoker. The E-flask was plugged with a smoke-tight, thick conical rubber plug whose central part has been hollowed out to allow for the passage of the hose into the E-flask. The smoke was pumped intermittently into the E-flask for 30 min. The resulting smoke–water was maintained as a stock solution in a refrigerator maintained at 0 °C, for latter use in the preparation of smoke–water of different dilution levels.

2.3. GA₃ and KNO₃ preparation

Stock solutions of 1 M concentrations from each of powdered GA₃ and KNO₃ (Sigma Chemical Company, Saint Louis, Mo, USA) were prepared using standard procedures. Concentrations of 0.1, 1, 10, and 100 µmol were prepared from each of the 1 M concentration of GA₃ or KNO₃ using the formula $C_1V_1 = C_2V_2$, where C_1 represents the 1 M solution; V_1 is the required volume of the solution from the 1 M stock solution; C_2 represents the desired concentration; and V_2 is the required volume of the concentration to be used for pretreating the seeds.

2.4. Effects of smoke–water, GA₃, or KNO₃ pretreatments

Sample seeds were drawn from the pooled seeds of the various provenances that were stored at 5 °C for 45 days. Healthy seeds were separated from the population by soaking the pooled seeds in a 5 l wooden-bowl half-filled with tap water. Seeds that floated were discarded, and those which sank were removed, blotted, and pretreated by soaking these in a 250 ml Erlenmeyer-flask containing 150 ml of the five different dilution levels of plant-derived smoke–water (i.e., 0, 1:1, 1:10, 1:100, and 1:1000) or the five different concentrations of GA₃ or KNO₃ (0, 0.1, 1, 10, and 100 µM). Pretreatments lasted for 6 h with continuous aeration made available by a small air pump. The air pump was previously optimized for providing just enough air such that the seed coats and endosperm did not become eroded. The control was set up using seeds pretreated with double distilled water only (i.e., zero concentration of the corresponding germination stimulant). A total of 6000 seeds was used for the smoke–water, GA₃, and KNO₃ experiments conducted under illuminated or non-illuminated conditions [i. e., 3 treatments * 5 different concentrations (including the control) * 2 light conditions * 10 replications per treatment * 20 seeds per replicate = 6000 seeds].

Twenty pretreated *C. macrostachyus* seeds were arranged in each of the 90 * 15 mm plastic Petri dishes overlaid with moistened Whatman # 1 filter paper. The seeds were allowed to germinate at ca 22 °C under either illuminated conditions (ca 60 µmol m⁻² s⁻¹ obtained from cool-white fluorescent lamps) or non-illuminated conditions (covered with ca 5–7 mm thick clean, sterile and moist sand, and kept away from the fluorescent light). Seeds were provided with an initial 5 ml of double-distilled water per Petri dish and, upon moisture depletion, were replenished with 2–3 ml of the same double-distilled water. A seed was considered germinated at the onset of radicle emergence for those germinated over filter paper. However, for those buried in sand,

appearance of the germinant as it pushes through the sand layer was considered as a criterion for successful germination. Germination counts were made every three days, and the experiments continued until at least 80% of the replicates from each of the treatments showed no further germination for three consecutive counts. Germination responses were expressed in terms of germination percentage (GP), mean germination time (MGT), and germination vigor (GV).

2.5. Effects of storage-time and temperature

Pooled seeds from the five provenances were maintained at 5, 15 and ca 22 °C (room temperature) for 1–8 months, and were subsequently used to study the effects of storage time and temperature on seed germination. Sample seeds were drawn every month from the respective storage conditions and were soaked and aerated for 6 h in a 250 ml Erlenmeyer-flask containing 150 ml of the smoke–water. Fifty smoke–water pretreated seeds were then planted in a conical pot (mouth diameter 200 mm, depth 200 mm) filled with clean sand. The seeds were covered with a layer of sand (ca 5–7 mm thick). The experiment was replicated 10 times. In total, 12,000 seeds were used in this study (3 temperature regimes * 8 storage periods * 10 replications * 50 seeds per replication = 12,000 seeds). Each pot containing the planted seeds was sprinkled with ca 1 l of tap water initially, and was provided with a daily dose of ca 500 ml thereafter. The pots were labeled and arranged at random on a wooden bench maintained in a glasshouse (RH 50–70%; temperature 25–28 °C). Dried grass stalks were used to cover the pots for conserving moisture, but the grass cover was removed as soon as the germinants started emerging to the surface of the sand. Seeds were considered germinated upon the emergence of germinants above the surface of the sand. Seed germination counts were made every three days, and germinants with two expanded leaves were removed and transplanted to potted soils. The experiment was discontinued when no further germination occurred for at least three consecutive counts. The germination responses of seeds were expressed in terms of germination percentage (GP), mean germination time (MGT), and germination vigor (GV).

Germination percentage (GP) was calculated according to the following formula:

$$1. GP = \left(\frac{n}{N}\right) \times 100\%, \text{ where:}$$

n	Number of germinated seeds;
N	Total number of seeds used per individual pretreatment

The mean germination time (MGT) and germination vigor (GV) were determined according to Labouriau and Agudo (1987) as follow:

$$2. MGT = \frac{\sum n_i t_i}{n}, \text{ where:}$$

n_i	Percentage of seeds germinated between two consecutive counts;
t_i	Time taken since germination experiment started;
n	Total percentage of seeds germinated.

$$3. GV = \sum \left(\frac{G_i}{t_i}\right) / N \times 100\%, \text{ where:}$$

G_i	Number of seeds germinated up to the day under consideration;
t_i	Time taken since the first day of incubation;
N	Total number of seeds.

2.6. Statistical analyses

Data analyses were performed by a one-way ANOVA using SPSS for windows version 12.0 with treatments considered as factors. Tukey

Honest Significant Difference Test was employed for the determination of significant differences among mean values of the various treatments. Mean differences between seed germination on Whatman # 1 filter paper and sand under the same treatment conditions were determined by using paired sample t-test. Unless stated otherwise, 5% significant level has been used to indicate statistically significant differences among treatments.

3. Results

Seed germination under laboratory conditions began 3 days after incubation and completed within 20–25 days in all the seeds pretreated with smoke-water (Fig. 1A, B). In contrast, germination began after an incubation period of 6 days for seeds pretreated with various concentrations of GA₃ or KNO₃ and lasted for over 32 days. Regular visual inspections showed that whereas seeds pretreated with GA₃, KNO₃, or distilled water (control) suffered from fungal attacks, those pretreated with smoke-water were free from such attacks (data not shown).

Compared to the control, seeds pretreated with smoke-water resulted in significantly ($P < 0.001$) higher final germination percentage whether or not these seeds were germinated under illuminated or non-illuminated conditions (Fig. 1A, B). However, the difference between seeds pretreated with smoke-water and the control was more marked in the illuminated (Fig. 1A) than in the non-illuminated seeds (Fig. 1B). Interestingly, seeds pretreated with smoke-water and maintained in darkness germinated significantly ($P < 0.001$) better

(over 85%), compared to the illuminated seeds (ca 60%) for similar seed lots and pretreatment procedures (Fig. 1A, B). Seeds pretreated with GA₃ or KNO₃ and germinated under no light conditions resulted in more than twice as much GP (66%), compared to those germinated under light conditions of 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (GP of only 30%).

Mean germination time (MGT) for seeds pretreated with smoke-water was ca 14 days, but increased significantly ($P < 0.01$) to ca 20 days for pretreatments involving GA₃, KNO₃, or distilled water (control) (Fig. 2). However, MGT values were rather similar for both the illuminated and the non-illuminated groups of seeds drawn from the same seed lots and with similar pretreatment dispensations.

Final germination percentage (GP) for seeds stored at 5, 15 and 22 °C at month 2 was quite high (up to 90%), and there was no significant difference among the three storage temperature conditions (Fig. 3). However, GP of seeds stored at 22 °C declined from a maximum of 88% (at month 2) to a minimum of 5% (at month 8), compared to those stored at 5 and 15 °C. After a storage period of 8 months, the maximum GP of seeds stored at 5 °C was ca 80%, only a 10% decline in germination percentage over a similar period of time, for similar seed lot, and identical pretreatment procedures (Fig. 3).

Similarly, mean germination time (MGT) was significantly influenced by storage periods of 1–8 months, and storage temperatures of 5, 15, and 22 °C; this increased significantly ($P < 0.01$) with increasing storage-time for all the three storage temperatures (Fig. 4). MGT for seeds stored at 5 °C was 22 days after a storage period of 6 months, while those stored at 22 °C for a similar extent of time required nearly twice as much time (a mean germination time of 38 days), showing

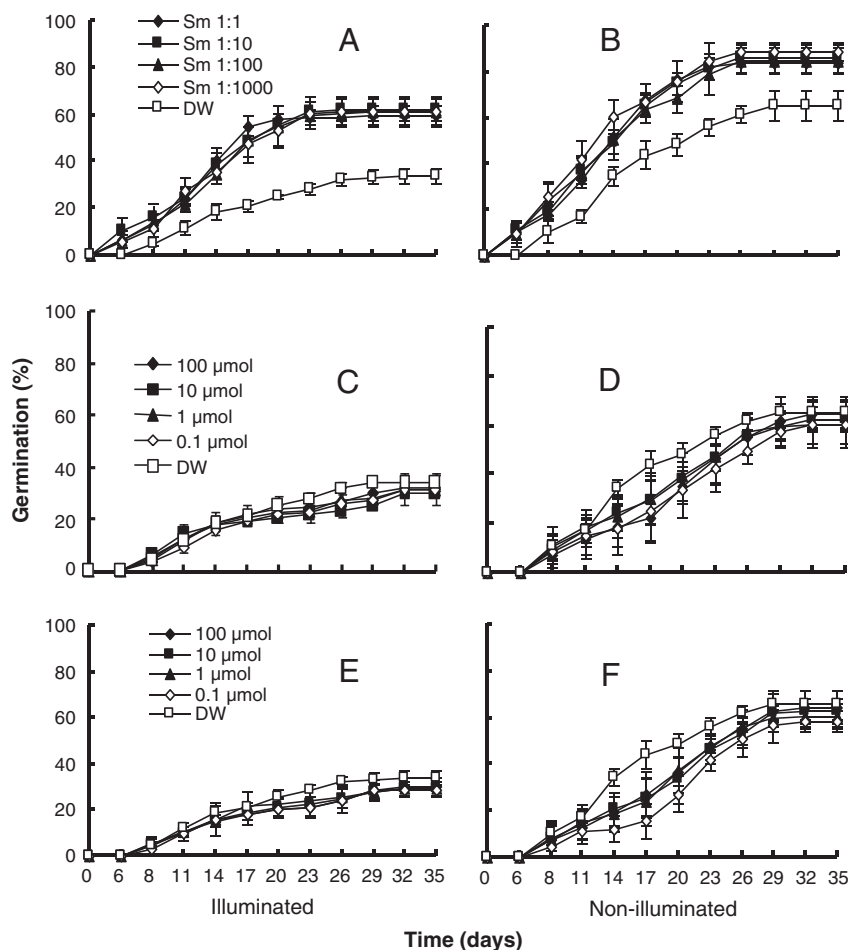


Fig. 1. Effects, on percentage germination of *C. macrostachyus* seeds, of smoke-water (Sm) in ratios of 1:1, 1:10, 1:100 and 1:1000 (A and B); GA₃ (100, 10, 1 and 0.1 μmol) (C and D); and KNO₃ (100, 10, 1 and 0.1 μmol) (E and F). Seeds were germinated on continually moistened Whatman # 1 filter paper under conditions of illumination (ca 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$; A, C, and E) or non-illumination in continually moistened sand (B, D, and F). DW = double distilled water treatment (control). Vertical bars indicate \pm SE (n = 10 replicates per treatment, with 20 seeds each).

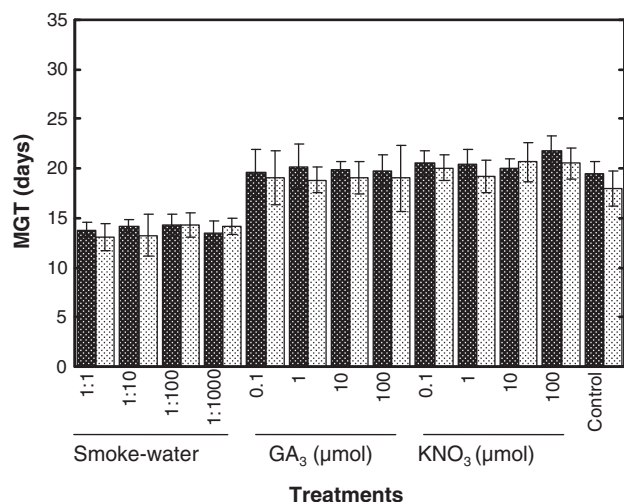


Fig. 2. Effects of various germination stimulants on mean germination time (MGT) of *C. macrostachyus* seeds. Seeds pretreated with the respective test solutions were germinated while buried in continually moistened clean sand (dark columns) or illuminated (ca $60 \mu\text{mol m}^{-2} \text{s}^{-1}$) on continually moistened Whatman #1 filter paper (light columns). Vertical bars indicate \pm SE ($n = 10$ replicates per treatment). The control was to pretreat seeds with double distilled water.

how much seeds of *C. macrostachyus* struggled to germinate when stored at room temperature and for a relatively extended period of time.

Germination vigor (GV) values of *C. macrostachyus* seeds stored at 5 and 15 °C were significantly ($P < 0.001$) higher than those stored at 22 °C, but vigor declined with storage-time for all the three storage temperatures (Fig. 5). GV dropped from 17% (at month 2) to only 4% (at month 8) for seeds stored at 22 °C. Similar but gentler drops were recorded for seeds stored at 5 and 15 °C: from 25% (at month 3) to 17% (at month 8); from 24% (at month 3) to 15% (at month 8), respectively (Fig. 5).

4. Discussion

Pretreatment of *C. macrostachyus* seeds with various dilution levels of smoke-water significantly improved germination percentage, mean germination time, and germination vigor compared to the control, GA_3 , or KNO_3 under both illuminated and non-illuminated conditions (Fig. 1). While the difference in germination response between the control and the smoke-water pretreated seeds was clear, there was no significant difference among the various dilution levels of the

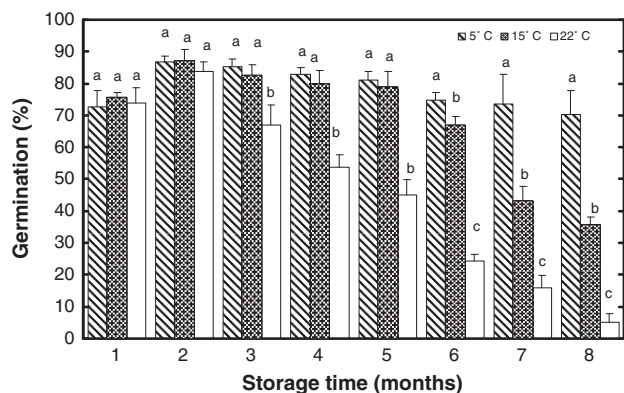


Fig. 3. Final germination percentage responses of seeds stored at 5, 15, and 22 °C for 1–8 months and pretreated with smoke-water at a level of 1:1000. For each storage month, columns bearing different letters are significantly different from one another. Bars on each column represent SE ($n = 10$ replicates per treatment, each with 50 seeds of *C. macrostachyus*).

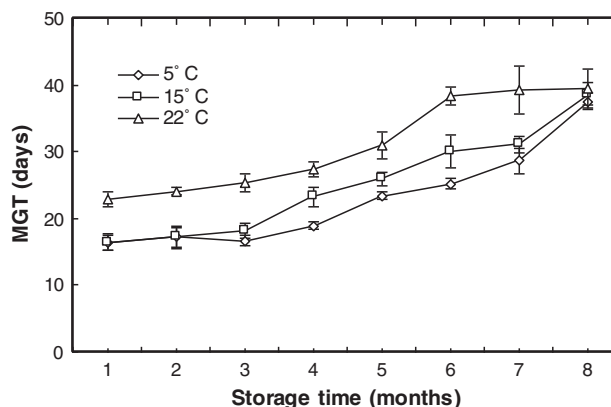


Fig. 4. Trends in the mean germination time (MGT) of *C. macrostachyus* seeds stored at 5, 15, and 22 °C for 1–8 months and pretreated with smoke-water at a level of 1:1000. Vertical bars indicate \pm SE ($n = 10$ replicates per treatment, 50 seeds per replicate).

smoke-water pretreatments (Fig. 1A, B). This study has clearly shown that *C. macrostachyus* seeds respond very well to the germination cue of smoke-water, more so under non-illuminated than under illuminated conditions.

The observation and report by Wicklow 1966 (quoted in Wicklow, 1977), Wicklow (1977), and the publication by De Lange and Boucher (1990) underpinned the importance of plant-derived smoke-water in increasing seed germination, as well as improvements in subsequent seedling development. This was substantiated by a relatively large numbers of studies (e.g., Brown et al., 2003; Crosti et al., 2006; Daws et al., 2008; Demir et al., 2012; Ghebrehiwot et al., 2011; Light et al., 2009; Roche et al., 1997; Van Staden et al., 1995, 2000). Significantly improved germination percentages of smoke-water pretreated seeds had been reported for many plant species from Australia (Bell, 1994; Bell et al., 1995; Dixon et al., 1995; Roche et al., 1997), as well as from South Africa (Brown et al., 1993; Pierce and Moll, 1994; Van Staden et al., 1995). Explanations for the underlying mechanisms of smoke-water-driven germination responses range from faster solute uptake by the germinating seeds (Keeley and Fotheringham, 1998; Light et al., 2002;) to those that invoke the presence of (an) active principle(s) in smoke (Baxter and Van Staden, 1994; Brown and Van Staden, 1997, 1999; Gardner et al., 2001; Keeley and Fotheringham, 1997; Keeley and Pizzorno, 1986; Light et al., 2002, 2009; Minorsky, 2002). A review by Light et al.

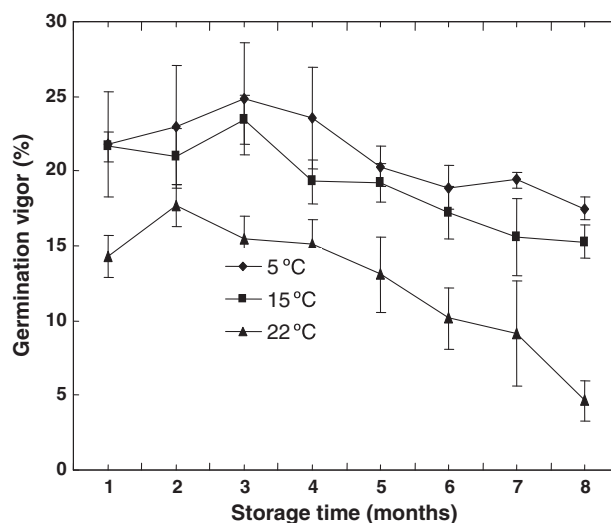


Fig. 5. Effects of storage time and temperature on germination vigor of *C. macrostachyus* seeds pretreated with smoke-water at a dilution level of 1:1000. Vertical bars represent \pm SD ($n = 10$ replicates per treatment, 50 seeds per replicate).

(2009) shed further light on the active principle believed to be responsible for seed germination of a variety of plants, including crops, weeds, as well as species from both fire- and non-fire-prone ecosystems. According to that review, research efforts of both South African and Australian scientists led to the characterization of a highly active butenolide compound, 3-methyl-2H-furo[2,3-c]pyran-2-one, from plant-derived smoke (Van Staden et al., 2004) and burned cellulose (Flematti et al., 2004 in Light et al., 2009). Butenolide is said to interact with gibberellins, and is alleged to have similar effects on germination as GA₃ by both stimulating and substituting for light in the germination of Australian Asteraceae. The compound is found not only to stimulate germination, but is also implicated in broadening environmental conditions over which germination can occur (Light et al., 2009, and the references therein).

Seed germination percentage and/or vigor of *C. macrostachyus* were significantly higher when these were germinated under non-illuminated than illuminated conditions, but extent of increase varied with pretreatment type: 70% of increase for a 1:1000 smoke–water pretreatment, compared to over 100% improvement for the GA₃ or KNO₃ pretreatment, suggesting different mechanisms for the two sets of smoke and GA₃ or KNO₃ pretreatments (compare Figs. 1A & B with C & E or D & F). Interestingly, germination responses of smoke–water pretreated seeds under illuminated conditions were comparable to those obtained for seeds pretreated with distilled water (control) and maintained under non-illuminated conditions (cf Fig. 1A & B).

It has been shown that light and temperature have strong inhibitory effects on germination percentage and vigor of some western Australian species, and that many small-seeded plant species germinated best under less fluctuating temperatures in darkness compared to those exposed to light (Plummer and Bell, 1995). The authors suggested that under fluctuating temperatures, light inhibition is the result of seed dormancy aimed at avoiding desiccation (and hence mortality) of emerging seedlings. Similar reports were made by Thanos and Georgiou (1988, cited in Bell et al., 1995) concerning the reduced germination percentage of some plant species from central Australia that were subjected to alternating day and night conditions. Chanyegna et al. (2012) studied germination responses and seed viability of the endangered *Widdringtonia whytei* Rendle and concluded that while temperature is one of the most critical factors for seed germination, the species does not require light to drive the latter process. On the other hand, it is known that exposure of seeds to light stimulates germination (e.g., Benvenuti et al., 2001; Brown and Van Staden, 1997, 1999; Drewes et al., 1995; Nishi et al., 2012; Thanos and Rundel, 1995; Thomas and Van Staden, 1995). Nishi et al. (2012) considered light as a critical cue for seed germination as well as for anisocotily in the small-seeded *Streptocarpus rexii* (Gesneriaceae); the authors characterized the species as photoblastic.

Most seeds of *C. macrostachyus* mature from December to February (i.e., during the prevalence of high diurnal temperatures and dry environmental conditions). Since the species is dry-deciduous (Negash, 2010), this is the period when large quantities of leaves are shed. Consequently, seeds shed to the ground should remain dormant under the thick layer of litter (i.e., in relative darkness), thus avoiding the adverse effects of direct sunlight and dry atmospheric conditions. Clearly, the superior germination response of *C. macrostachyus* under non-illuminated than under illuminated conditions may relate to a useful ecological adaptation of the species. It has been reported that seeds of some South African and western Australian plant species escape death by remaining dormant under the thick layer of litter produced from the shed leaves of the mother tree (Bell, 1994; Benvenuti et al., 2001; De Lange and Boucher, 1993). A related, but not identical, issue is the soil seed bank where seeds of certain plant species require a period of after-ripening during which they interact with the soil components before germination occurs (Keeley and Fotheringham, 1998). Such seeds can stay dormant in the soil and readily germinate as soon as they get appropriate germination conditions (Cone and Kendrick, 1986). Since soil temperature

under shady conditions is relatively lower and more constant than air temperature, soil seed bank has a very important ecological advantage (Baskin and Baskin, 1989). Negash (1995, 2010) noted that seasonal synchrony in the phenology of native trees has important role as a regulatory mechanism for seed germination from soil seed bank. When changes occur in the seed environment as a result of soil disturbance and/or removal of canopy, seeds may be responsive to conditions that promote germination.

Compared to the control, GA₃ or KNO₃ failed to effect significantly higher germination percentage (GP) in *C. macrostachyus*, both under illuminated and non-illuminated conditions (Fig. 1C–F). Further, GA₃ and KNO₃ were practically similar in their effect on GP across all ranges of concentrations: maximum of 30% under illuminated (Fig. 1C, E), and 60% under non-illuminated (Fig. 1D, F) conditions. The fact that the two classical germination stimulators failed to improve germination response of *C. macrostachyus* indicates that its seeds are non-photoblastic (i.e., light is not obligatory for germination). It has been reported that seeds of plant species whose germination indices get improved by GA₃ or KNO₃ are mostly those that require light for germination (Alboresi et al., 2005; Hartmann et al., 1997; Taiz and Zeiger, 1998). For example, when seeds of *Paulownia tomentosa* L. (a species whose seeds require light for germination) were germinated under light conditions alone, they attained similar percentage germination as those treated with GA₃ and germinated in darkness (Jovanovic et al., 2005). Also, in several native Australian Everlasting Daisies (Asteraceae, Tribe Inuleae), application of GA₃ (50 mg l^{−1}) in darkness overcame the light requirement and stimulated seed germination to similar levels observed in light-treated seeds (Plummer and Bell, 1995). Previous studies reported that KNO₃ enhances germination in certain plant species such as *Sisymbrium officinale* (L.) Scop., *Arabidopsis thaliana* (L.) Heynh. and *Emmenanthe penduliflora* Benth. (Derckx and Karssen, 1993; Hilhorst and Karssen, 1988; Keeley and Fotheringham, 1997; Minorsky, 2002). It had also been noted that GA₃ or KNO₃ become effective germination-promoting agents when combined with light and appropriate temperatures (Bewley and Black, 1994; Thanos and Rundel, 1995). In contrast, our study showed that presence of light reduced germination percentage of *C. macrostachyus* by half (from over 60 to 30%; Fig. 1C–F), thus demonstrating the overriding effect of light on GA₃ or KNO₃.

It is generally known that different native plant species develop different survival strategies depending on the environment under which they have evolved (Negash, 1995, 2003, 2010). For example, tree species such as *Acacia abyssinica* Benth., *Podocarpus falcatus* Thunb. (Mirb.), and *Olea europaea* L. subsp. *cuspidata* (Wall. ex DC.) Ciffleri are adapted to environments with alternating short and long rainy and dry seasons, respectively. Consequently, these trees produce seeds that possess hard and/or woody seed coats, thus surviving the dry season by protecting their embryos from desiccation (Negash, 1992, 1993, 1995, 2010). Similarly, seeds that remain dormant under the thick layer of litter produced from the leaves of mother trees escape desiccation and/or seedling mortality during the hot and dry season (Benvenuti et al., 2001). In this regard, *C. macrostachyus* probably follows the latter strategy for its continued survival.

Storage temperature had significant influence on germination indices of *C. macrostachyus* seeds. The rapid decline in percentage germination and reduced germination vigor during seed storage at room temperature (ca 22 °C) indicate that higher temperatures are injurious to seeds of *C. macrostachyus*. Our results (Figs. 3–5) show that this is indeed the case. However, scrutiny of these same results reveals possibilities for increasing the longevity of seeds by maintaining them at suitable temperatures. Keeping seeds for 8 months at 5 °C, for example, preserved seed viability and percentage germination better than those stored at 15 or 22 °C (cf percentage germination of 70% for seeds stored at 5 °C with percentage germination of 38 and 5% for seeds stored at 15 and 22 °C, respectively). From the present studies, we conclude that propagation of *C. macrostachyus* by seed is relatively

easy and fast, and that pretreatment of seeds with smoke–water is cheaper, technically less demanding and also yields much better germination results, compared to GA_3 or KNO_3 pretreatments. It is further concluded that *C. macrostachyus* seeds are intermediate between orthodox and recalcitrant seeds, and that they are non-photoblastic.

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